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Modulating the oxidation of cucurbit[*n*]urils

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The functionalisation of cucurbit[*n*]uril macrocycles carried out through an oxidative approach in water using ammonium persulfate was studied. Through complexation with a doubly-charged bisimidazolium guest we were able to detect, distinguish and quantify the presence of each CB[*n*]-$(OH)_{\mathbf{x}}$ (where $1 \leq x \leq 2n$) derivative for the first time. The impact of oxidation on each CB[*n*] (n = 6 – 8) was studied individually, as well as in the presence of other competing CB[*n*] species. We were able to understand the reactivity of the parent CB[*n*] alongside its hydroxylated derivatives, CB[*n*]-$(OH)_{\mathbf{x}}$, and show that the oxidation of CB[*n*] through a free-radical approach cannot result in stoichiometric hydroxylation despite previous literature reports by Bardelang, Ouari and co-workers, JACS, 2015, **137**, 10238. Furthermore, an in-depth study on hydroxylation of CB[7] was conducted. Through DFT calculations we were able to show that the second hydroxy substituent is preferentially located on the same glycoluril unit. Moreover, through optimisation of the reaction conditions we were able to access and a protocol for the controlled oxidation to yield a chemically monofunctional CB[7] derivative.

Cucurbit[*n*]urils, CB[*n*], are an emergent class of macrocylic host molecules formed from the cyclisation of n glycoluril (n = 5 – 8, 10) units joined by methylene bridges.[1,2] CB[*n*] have shown superior recognition and binding properties compared to other families of macrocyclic host molecules, such as cyclodextrins or calixarenes.[3] They have a symmetric and rigid structure with a well defined cavity, that is capable of encapsulating guest molecules with binding constants up to 10^{15} M^{-1},[4,5] which is on the same order of magnitude as those found in nature by biotin-avidin pairs.[4,5] The affinities of guests for CB[*n*] span a range greater than ten orders of magnitude, giving these macrocycles a unique position as hosts on account of the selectivity and strong binding they can offer within supramolecular systems.[6] In recent years, CB[*n*] have been applied in many different fields,[3,7] however, in order to fully exploit these macrocycles in more complex and designed systems, derivatisation of CB[*n*] has been an ongoing effort for the past two decades.[3] The lag time between the discovery and first report of CB[6] in 1905,[8] characterisation in 1981,[1] isolation of further CB[*n*] homologues (n = 5, 7 – 8) in 2000[2] and functionalised derivatives emphasises the difficulty of the task. Indeed, the first functionalised CB[*n*] derivative was only reported in 1992 by Stoddart and co-workers.[9]

The introduction of any functionality on the periphery of CB[*n*] has been a formidable task within the field,[10] hampered by the rigid CB[*n*] structure, poor solubility and the limitation of only C–H reactive sites available for activation. As CB[*n*] has a robust chemical structure that is resistant to both strong acids and bases, only a limited range of chemical modifications can be successfully applied to the CB[*n*] structure. The addition of a single functionality to the periphery of the CB[*n*] to form a monofunctionalised derivative is of particular interest. This would allow for a high degree of control for conjugation to surfaces or other relevant constructs, especially for the larger CB[7] and CB[8] homologues, which exhibit much richer guest binding profiles.[5]

A direct oxidative functionalisation approach, where the parent CB[*n*] is oxidised to access monofunctional CB[*n*] derivatives, has seen the most success with several recent reports.[11–13] However, as first identified by Kim and co-workers[5,14], the monofunctionalisation of cucurbit[*n*]uril macrocycles through straightforward free–radical oxidation is difficult to control. An oxidative approach with potassium persulfate (KPS) was first reported in 2003 to form the perhydroxylated CB[6]-$(OH)_{12}$.[14] Scherman and co-workers augmented this method and utilised solubilising agents such as bisimidazolium compounds to increasing the concentration of the reaction mixture. The augmented method included the use of ammonium persulfate (APS) and they consequently reported the first fully-characterised, monofunctional CB[6] derivatives including a guest-free CB[6]-$(OH)_1$ crystal structure.[11] Isaacs and co-workers developed a method for the synthesis of an open chain hexamer, which was shown to undergo cyclisation with functionalised glycolurils to yield functionalised CB[*n*] derivatives.[15] As the isolation of large amounts of pure, open-chain oligomers requires ample synthetic skill, it is not surprising that alternative approaches have been explored. Bardelang, Ouari and co-workers reported the direct, quantitative formation of monofunctionalised CB[*n*] (n = 5 – 8) using a free-radical oxidation with hydrogen peroxide, initiated with light.[13] Moreover, the challenge of accessing pure, monofunctionalised

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derivatives was further confirmed by these results describing the quantitative conversion of CB[*n*] to CB[*n*]-$(OH)_1$ being recently revisited; the co-authors have now substantially corrected their claims.[16] Herein we report the reactivity of CB[*n*] under oxidative conditions and show that accessing monofunctionalised derivatives through a straightforward oxidative free radical approach is not possible. The oxidation of CB[*n*] as part of a complex mixture hinders the oxidation of the larger CB[7] and CB[8] homologues. Furthermore, this approach leads to the formation of mixtures containing derivatives with only one or two hydroxy functionalities. We are able to show that the second hydroxy functionality has a significant preference to form on the same glycoluril unit as the first, which allows for effective "monofunctionalisation" through the controlled oxidation of CB[7] and CB[8]. Functionalised derivatives have also been further modified to allow immobilisation onto surfaces[12,14] and attachment to polymeric backbones has been shown by Tan and co-workers.[17] As the isolation of large amounts of pure, open-chain oligomers requires ample synthetic skill, it is not surprising that the oxidative approach of the parent CB[*n*] homologues has been more widely explored. This method relies upon the formation of OH radicals leading to hydroxylation at the equatorial C–H positions. Herein, we focus on understanding the reactivity of each CB[*n*] (n = 6 – 8) homologue towards oxidation. Depicted in Figure 1 is an overview of the approach taken in this study. APS was employed as a source of OH radicals throughout, as it does not require the use of an external UV light source, has been shown to be successful in the oxidation of CB[6][11], and, importantly, makes analysis by ESI-MS more straightforward resulting in spectral clarity.

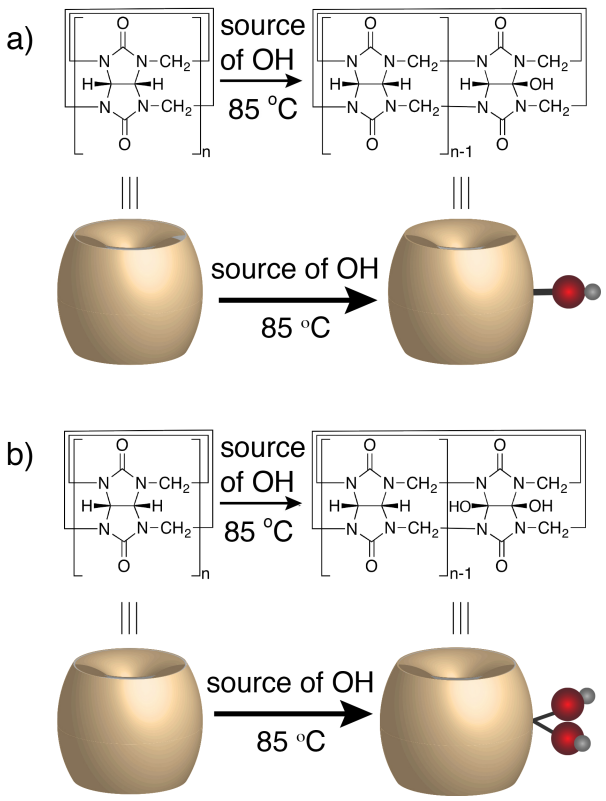

Fig. 1 Schematic of CB[*n*] structure and its modulation through the introduction of a hydroxyl group at the equatorial position.

1 Results and discussion

1.1 Experimental considerations

In order to access monofunctional derivatives in a more quantitative, controlled manner, initial experiments were aimed at gaining critical insight into the reactivity of each CB[*n*] homologue.Radical reactions are not often easy to control, especially on macrocyclic molecules made of identical repeating units, such as CB[*n*]. Furthermore, monitoring the oxidation of CB[*n*] is not straightforward as there are few techniques with suitable sensitivity to accurately distinguish and detect CB[*n*] from CB[*n*]-$(OH)_x$ species, or more importantly, the CB[*n*]-$(OH)_x$ species from one and another. Commonly employed techniques, such as ^{1}H and ^{13}C NMR, cannot be used to fully understand the substitution pattern or quantify the distribution of CB[*n*]-$(OH)_x$ products. The signals of the OH substituent are not observed due to fast exchange in D_2O and those for adjacent protons are unable to quantify the abundance of further oxidation products, CB[*n*]-$(OH)_x$ $1 \leq x \leq 2n$ due to overlapping resonances. Electrospray ionisation mass spectrometry (ESI-MS), has seen increasing use in the characterisation of supramolecular systems for which other techniques lacked the sensitivity required for full and detailed characterisation.[18,19] This technique is now regarded as an important analytical tool within the field.[20] In this study ESI-MS was employed to probe the reactivity of the CB[*n*] homologues and quantify the distribution of the homologues and further oxidation products present in solution.

1.2 Solubility limitations of CB[*n*]

One of the major limitations of CB[*n*] chemistry, and consequently one of the biggest hindrances to functionalisation, is the largely insoluble nature of CB[*n*]. All CB[*n*] homologues are sparingly soluble in aqueous environments. For CB[6] and CB[8] however, the aqueous solubility is extremely poor, typically 0.1 mM for CB[6] and <0.1 mM for CB[8].[21] In contrast, CB[5] and CB[7] show enhanced solubility of up to 20 – 25 mM.[21] CB[*n*] homologues are insoluble in organic solvents, therefore traditional organic transformations on parent CB[*n*] are not possible. Furthermore, the functionalisation is essentially a C–H activation, yet the strong propensity of CB[*n*] to bind metal cations prohibits stoichiometric, metallic based C–H activation methods. Consequently, the oxidative radical method reported by the groups of Kim[14] and Scherman[11] has shown the most success. Nevertheless, solubility remains problematic. It was reported by Scherman[22] and others[23,24] that the solubility of CB[*n*] homologues, (n = 6) is greatly enhanced by the encapsulation of an imidazolium based ionic liquid guest molecule inside the cavity of the macrocycle. Reported in the synthesis of monofunctionalised CB[6][11], a bisimidazolium guest was used to greatly enhance solubility and promote functionalisation. This guest has also been used in the analogous functionalisation of CB[7] by Tan *et al.*[17]

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1.3 ESI-MS as an analytical tool for the detection and identification of CB[*n*] and derivatives in complex mixtures

ESI-MS was utilised in this study to analyse the CB[*n*] oxidation reaction mixtures. However, on account of their bulky structure and chemically-inert nature, both of which make ionisation difficult, CB[*n*] alone do not ionise well. The presence of additional cations such as K^+ or Na^+ is normally required to increase their propensity to fly but detection is not always facile. For the spectra obtained from ESI-MS analysis to be representative of the bulk solution composition, we had to circumvent the poor ionisation of CB[*n*] and did so through the formation of CB[*n*]·guest complexes. The use of bisimidazolium guest moieties, whose affinities range between 10^4 to 10^9 M^{-1} [23], allowed for the quantitative detection of the supramolecular complexes, and thus the macrocyclic homologues, by ESI-MS. Furthermore, these guest moieties have a double charge and once encapsulated in CB[*n*], these doubly-charged host·guest complexes, both pre- and post-functionalisation, are readily detected by ESI-MS. The CB[*n*] complexes ionise on account of the guest moiety and in all cases observed throughout this study, no peaks for any guest-free species were ever detected, either as cations themselves or as commonly observed K^+ or Na^+ adducts. This suggests that the introduction of functionality on the periphery of CB[*n*] does not affect the detection of CB[*n*] host·guest complexes by ESI-MS. The encapsulation of bisimidazolium guest moieties by CB[*n*] therefore has a dual-purpose in this study, to enhance the solubility during the oxidation reaction and to allow CB[*n*] and hydroxylated derivatives to be quantitatively detected by ESI-MS.

1.4 Oxidation of CB[*n*] species

Initially the oxidation of each CB[*n*] was studied. A bisimidazolium guest with an eight carbon alkyl linker (**G1**) was used in the oxidation of all CB[*n*] as it can readily complex all homologues studied. Binding constants (K_a) measured with isothermal titration calorimetry (ITC) are reported in Figure 2, the value for CB[6] has been previously reported [11] (see Supporting Information Figures S1a-b for ITC curves of **G1** to CB[7] and CB[8]).

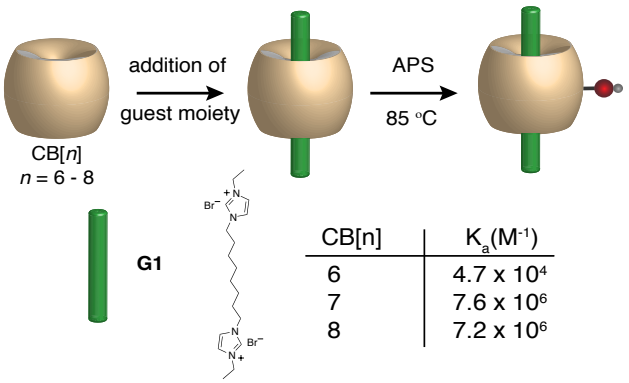

Fig. 2 Complexation of **G1** with CB[*n*] prior to oxidation and binding constants measured using ITC.

1.4.1 Oxidation of CB[*n*] as solo events.

Each CB[*n*] (n = 6 – 8) was reacted with one molar equivalent of APS and the oxidation monitored using ESI-MS. Using the imidazolium guest we were able to successfully visualise the doubly-charged host·guest complexes present in the reaction mixture (see Figures S5–7) For each CB[*n*] homologue, the oxidative reactivity was found to be different as can be seen in Figure 3. Conversion of CB[*n*] to functionalised CB[*n*] (f-CB[*n*]) decreased with increasing homologue size. For CB[6], (Figure 3a) prior to the crossover point at 275 min a higher concentration of non-reacted CB[6] compared to functionalised CB[6] (f-CB[6]) was observed. At no point do the lines appear to plateau suggesting that the oxidation reaction continues after 24 h. In the case of CB[7] and CB[8], however, no crossover point is observed. The predominant component of the mixture remains unreacted parent CB[*n*] homologue, even after 24 h. Furthermore, CB[7] appears to plateau at 660 min and CB[8] much sooner at only 120 min. If the reactivity of CB[*n*] with APS was stoichiometric and only related to the concentration of OH radicals present in solution then the abundance of CB[*n*] would be expected to continue decreasing until it was completely consumed. This is not observed as illustrated in Figure 3. The decrease in conversion of CB[*n*] to f-CB[*n*], therefore, cannot be related to the concentration of OH radicals and likely stems from an intrinsic property of the parent CB[*n*] structure.

A probable explanation for this difference in conversion is the bond dissociation energy (BDE) of the C–H equatorial bond. Bardelang, Ouari and co-workers [13] reported DFT calculations for the BDE of the C–H equatorial bond in CB[*n*] (n = 5 – 8) homologues. Based on the reasonable assumption that all C–H bonds in the parent CB[*n*] are equivalent, they reported that the BDE of the C–H equatorial bond increases between the CB[*n*] (n = 6 - 8) homologues. Therefore, the energy required for hydrogen abstraction at the equatorial C–H position (to form a tertiary CB[*n*] radical species) increases with increasing homologue size. The lower conversion of CB[*n*] to f-CB[*n*] in larger homologues can therefore be explained by this difference in BDE.

Using ESI-MS, we were able to quantify not just the reactivity of each CB[*n*] but also the distribution of the oxidation products. There was a range of oxidised derivatives observed in all cases with varying numbers of substitutions on the CB[*n*]. CB[6] showed the largest conversion to f-CB[6] (Figure 3a) and consistent with this observation, CB[6] also showed the widest range of oxidised derivatives (Figure S6 a) with up to five hydroxy substitutions detected. With increasing homologue size, CB[7] and CB[8] showed a narrower range of substitutions (Figure S6 b-c). Small quantities of CB[8] derivatives bearing a maximum of only three hydroxy functionalities were detected after 24 h. Mixtures composed of only unreacted CB[*n*] and CB[*n*]-$(OH)_1$ were never detected for any CB[*n*] homologue at any measurable reaction time ($t > 30$ s). Despite equimolar quantities of CB[*n*] and APS, stoichiometric conversion of CB[*n*] to CB[*n*]-$(OH)_1$ was not observed. This is on account of the intrinsic reactivity of CB[*n*] (Figure 3) and the difference in reactivity between CB[*n*] and f-CB[*n*]. The structure of CB[*n*] contains many ($2n$) chemically-equivalent reaction sites, none of which are rendered non-reactive by the in-

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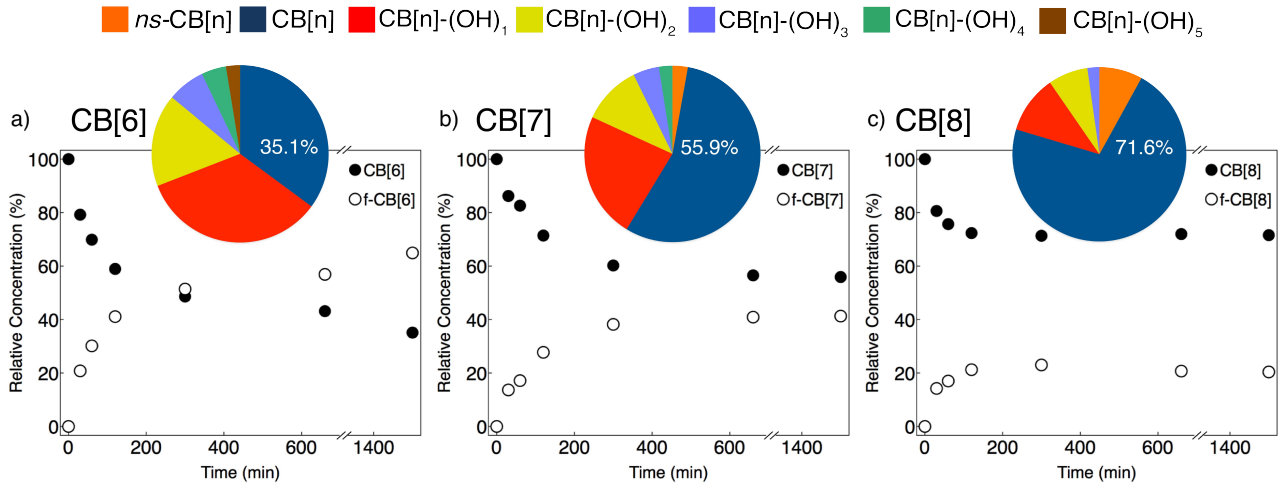

Fig. 3 Graphs a - c show the depletion of CB[*n*] (filled circles) and the formation of f-CB[*n*] (unfilled circles) as a function of reaction time for each CB[*n*] ($n = 6 - 8$). Values calculated are based on the abundance of each CB[*n*] as a host·guest complex with bisimidazolium guest **1** detected using ESI-MS.

troduction of a single functionality. An OH radical is unable to chemically distinguish between a C–H on a CB[*n*] or a f-CB[*n*]. Therefore, a quantitative stoichiometric conversion of CB[*n*] to CB[*n*]-$(OH)_1$ is not observed in agreement with the reports of fully-functionalised derivatives, CB[*n*]-$(OH)_{2n}$ ($n = 6 - 8$), in the literature.[14]

This data contradicts the initial report by Bardelang, Ouari and co-workers[13] where a high conversion to pure monohydroxylated CB[*n*]-$(OH)_1$ *via* an oxidative OH radical approach was initially suggested. Furthermore, for the derivatives with more than one hydroxy substitution, the possibility of constitutional isomers increases the complexity of the mixtures obtained. These constitutional isomers cannot be distinguished using ESI-MS alone. It should be noted that for all CB[*n*] shown in Figure S6 the abundance of each CB[*n*]-$(OH)_x$ derivative present in the mixture decreases upon further hydroxylation. This supports a step-wise oxidation mechanism compared to a runaway, random oxidation. CB[*n*]-$(OH)_1$ is formed first from CB[*n*] and immediately leads to the formation of CB[*n*]-$(OH)_2$ from CB[*n*]-$(OH)_1$ before complete transformation from CB[*n*] to CB[*n*]-$(OH)_1$.

1.4.2 Oxidation of CB[*n*] as a complex mixture.

To attempt to modulate and further investigate the reactivity, a competitive oxidation was trialled where equimolar amounts of CB[*n*] ($n = 6 - 8$) underwent oxidation as a part of a competitive mixture with 3 molar equivalents of APS oxidant. As depicted in Figure 3, CB[*n*] and their respective oxidised derivatives (CB[*n*]-$(OH)_x$) could be detected and quantified using ESI-MS. Increasing the complexity of the mixture, *i.e.* the presence of other CB[*n*] homologues, did not change the trends in conversion of CB[*n*] to f-CB[*n*]. It did, however, result in a decrease in the overall conversion of each CB[*n*], which might suggest an interesting route to limiting the number of hydroxy substituents. For CB[*n*], under both 'solo' conditions and within a complex mixture, a range of oxidised derivatives were formed. In the presence of other competing CB[*n*] species, the oxidation of CB[*n*] appeared to become more controlled resulting in a decreased amount of oxidised derivatives and a narrower range of derivatives produced for all CB[*n*].

The formation of products observed in ESI-MS with m/z peaks of 6 less than the original CB[*n*]·G1 complex were detected for CB[7] and CB[8] under both 'solo' and complex oxidation conditions. As evident in Figure S6 a-f, formation of this product is more prevalent with the larger CB[8] homologue and could also be observed with CB[7] at longer reaction times but never for CB[6]. We propose that this peak corresponds to a degradation product of the parent CB[*n*] (*deg*-CB[*n*]), formed when CB[*n*] is exposed to long reaction times and elevated temperatures (Figure S2). Through controlling the reaction time to < 120 min, the formation of *deg*-CB[7] can be avoided without impacting the yield of f-CB[7] derivatives. Conversely, modulation of the reaction time for CB[8] under both 'solo' and competitive conditions is not able to prevent degradation. On account of the complexity of the competitive mixture, CB[8] (Figure S6 f) showed both enhanced degradation, but also resulted in a narrower range of functionalised derivatives with only CB[8]-$(OH)_1$ and CB[8]-$(OH)_2$ detected. This enhanced selectivity allows more controlled access to f-CB[8] derivatives.

Although stoichiometric conversion through an oxidative free radical approach is not possible, it is clear that the reactivity of CB[*n*] can be modulated to yield mixtures composed of more favourable ratios of f-CB[*n*] for subsequent transformations. In particular, the ability to access mixtures with a limited number of hydroxy substituents was of great interest. It was clear that the reactivity could be modulated through the introduction of competitive species however, for the larger CB[7] and CB[8] derivatives this did result in degradation. Moreover, this approach would still require the separation of the f-CB[*n*] from the mixture which would not allow access to functionalised derivatives in a scaleable facile manner. We therefore decided to undertake a detailed study on the oxidation of CB[7], probe the factors affecting oxidation and investigate ways in which the reactivity of the parent CB[7] could be modulated.

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1.5 Oxidation of CB[7]

CB[7] displayed a moderate distribution of oxidation products, Figure 3 and S6, and the formation of *deg*-CB[7] could be avoided by limiting the reaction time.

1.5.1 Addition of a guest moiety *pre* or *post* oxidation.

Although a guest moiety was required for the detection of CB[*n*] and its derivatives, it was not clear if the formation of the host·guest complex before oxidation had any significant effect on the reaction. The oxidation was trialled both adding the guest prior to oxidation and after. On account of the higher aqueous solubility of CB[7] compared to CB[6] and CB[8], the oxidation under guest-free conditions could be carried out at a higher concentration (20 mM) but for consistency it was carried out at 1 mM. A

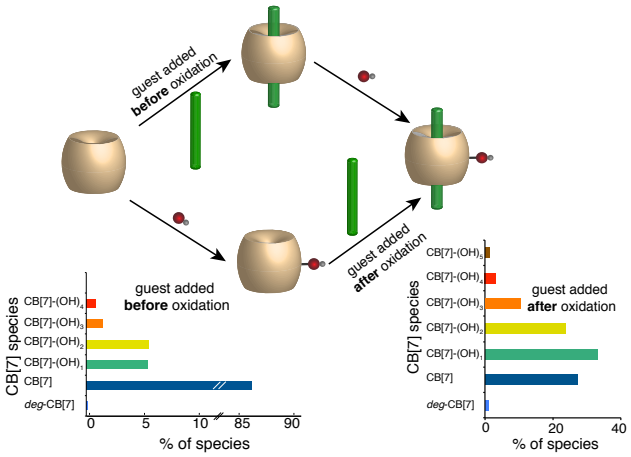

Fig. 4 (a) Schematic representation of two guest complexation strategies prior to ESI-MS analysis. (b) Representative ESI-MS spectra showing the difference in the abundance of the CB[7] and f-CB[7] species following 30 min reaction time adding the guest pre– or post–oxidation.

guest-free approach towards oxidation led to significantly higher conversion of CB[7] to f-CB[7], (72.8% conversion guest-free *v.s* 13.8% with **G1** present), Figure 4. Guest-free oxidation also led to a wider range of oxidised derivatives; within a 30 min reaction time, derivatives with up to five hydroxy substitutions were detected. It was evident that the presence of the guest moiety during the oxidation resulted in a more controlled reaction yielding a narrower distribution of oxidation products. Despite having a significantly lower conversion of CB[7] to f-CB[7] a guest-free approach is not ideal considering the associated difficulty in separating the further oxidation products.

1.5.2 Oxidation with different guest moieties.

Following on from probing the impact of having a guest present we decided to vary the structure of the guest. A variety of guest molecules were studied, Figure 5 and the impact of these moieties on the distribution of f-CB[7] formed was assessed (Figure S7).

All guest molecules were reported to bind 1:1 to CB[7] with moderate to strong binding constants detailed in Figure 5.[23,25,26] ESI-MS experiments clearly indicate that the guest does not participate in the oxidation reaction, yet the choice of guest clearly has an impact on the distribution of oxidised derivatives formed,

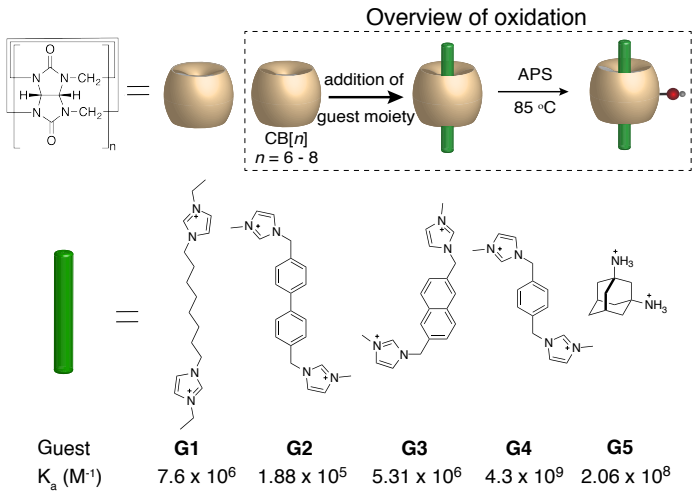

Fig. 5 Structures of guests used for CB[7] oxidation with previously reported binding constants (K_a) for **G2**–**G5**;[23,25,26] the binding constant of **G1** with CB[7] has not been previously reported and the ITC curve is shown in Figure S1a. All counterions used were Br^-, except for **G5**, which is Cl^-.

Figure S7. The differences in the distribution of oxidised derivatives must arise on account of the change in reactivity of CB[7] upon the encapsulation of different guest species. The binding of larger guests has been reported to cause ellipsoidal deformation of the CB[*n*] cavity and is evident (in both solid state and solution) through x-ray crystallography[27,28] and Raman spectroscopy.[29] It is therefore likely that these changes in reactivity are a result of a change in the BDE of the equatorial C–H bonds as a result of the structural deformation caused through encapsulation of a guest moiety. It is clear from comparing the conversion of CB[*n*] $n = 6 - 8$ (shown in Figure 3) that a subtle change in the BDE of the C–H equatorial bonds can result in a marked change in the conversion of CB[*n*] to f-CB[*n*]. Each guest, **G1**–**G5**, resulted in a different conversion of CB[7] to f-CB[7] and a different distribution of oxidation products.

From the results shown in both Section 1.5.1 and 1.5.2 it is clear that not only is there is a marked effect on the conversion of CB[*n*] to f-CB[*n*] when a guest is present but the choice of guest allows some ability to modulate the distribution of the oxidation products. This understanding into the reactivity has not been shown previously and moreover, the ability to modulate reactivity and offer enhanced control over CB[*n*] has, to date, remained unexplored. These results prompted us to try to control the distribution of CB[7] oxidation products such that the maximum conversion to CB[7]-$(OH)_1$ and CB[7]-$(OH)_2$ was achieved whilst avoiding degradation. The degradation of CB[7] did not occur in the presence of **G1** or **G2**, as overall conversion to CB[7]-$(OH)_1$ was highest with **G1**, it was used for further investigations.

The swift formation of CB[7]-$(OH)_2$ from CB[7]-$(OH)_1$ prompted us to investigate why its formation could not be avoided through limitation of oxidant or extremely short reaction times (30 s). This suggested that either a stabilising effect or C–H bond activation on account of a proximate OH substituent promoted the second oxidation. We therefore calculated the energies

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of the CB[7], CB[7]-$(OH)_1$ and 13 resulting possible CB[7]-$(OH)_2$ structures with the second OH located at equatorial positions 1–13 as depicted in Figure 6.

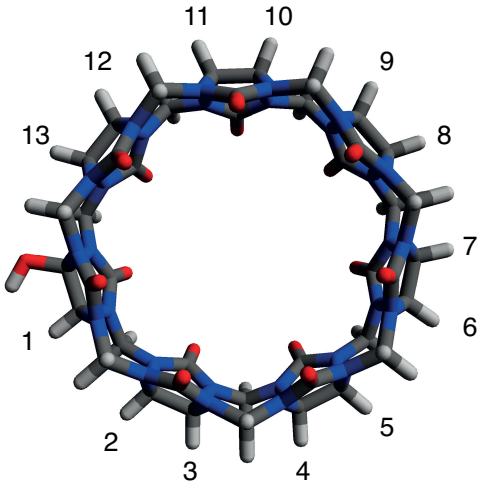

Fig. 6 Schematic showing the DFT (B3LYP-D3/6-31+G*) calculated CB[7]-$(OH)_1$ structure and highlighting the 13 different sites for the introduction of a second OH group to yield CB[7]-$(OH)_2$.

Interestingly, we found that the energies of the various CB[7]-$(OH)_2$ structures differed by 0.6 kcal/mol depending on whether the second OH was on the same glycoluril unit as the first one, or on any of the other six units, Table S1. Indeed, continuum solvent[30] DFT (B3LYP-D3/6-31+G*)[31,32] geometry optimisations using the Gaussian program[33] showed that the lowest energy CB[7]-$(OH)_2$ structure was the one in which both OH substituents were located on the same glycoluril unit. This thermodynamic preference offers some insight into the reason behind the quick formation of CB[7]-$(OH)_2$ under oxidative conditions and the ability to control the oxidation to yield solely CB[7]-$(OH)_1$. In all experiments, the observation of CB[7]-$(OH)_1$ using ESI-MS was concomitant with the appearance of CB[7]-$(OH)_2$. There was no accumulation of CB[7]-$(OH)_1$ before CB[7]-$(OH)_2$ was observed and the abundance of both species grew with extended reaction time.

With this novel insight into the substitution pattern of CB[7]-$(OH)_2$ we aimed to optimise the oxidation of CB[7] and limit the formation of CB[7]-$(OH)_x$ (whereby $x \geq 3$). As depicted in Figure 7, we were able to successfully modulate the reactivity of CB[7] through equivalents of oxidant and short reaction time to yield a mixture containing 64.3% CB[7], 30.9% CB[7]-$(OH)_1$ and 4.8% CB[7]-$(OH)_2$, without any higher oxidation products. Formation of a CB[7]-$(OH)_2$ with the second OH substituent located on the same glycoluril unit renders it as a 'monofunctional CB[7]' entity for further derivitasation as, on account of sterics, only one OH moiety can undergo further transformation. Thus, after subjecting CB[7] to oxidative conditions for a short reaction time (<1 h) we achieve a reproducible mixture of 64.3% CB[7] and 35.7% 'monofunctional' CB[7], which can be used directly in subsequent transformations and can be scaled accordingly.

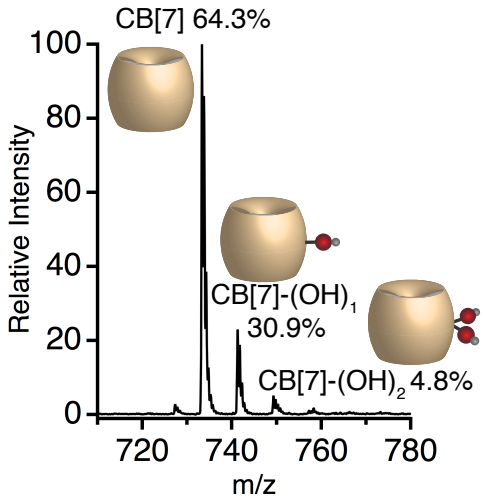

Fig. 7 Schematic showing the optimised oxidation of CB[7] to produce a mixture containing CB[7]-$(OH)_1$ and CB[7]-$(OH)_2$ only.

2 Conclusions

We have presented a strategy for the detection and quantification of CB[*n*] and oxidised derivatives as host-guest complexes in solution using ESI-MS. The ability to detect the f-CB[*n*] derivatives and distinguish and further quantify their abundance based upon the number of hydroxy substitutions has not been previously reported, which likely led to misdrawn conclusions by Bardelang, Ouari and co-workers. Using this methodology, the reactivity of each CB[*n*] has been explored and found to differ between homologues. The conversion of CB[*n*] to f-CB[*n*] is likely based upon the BDE of the C–H equatorial bond and roughly independent of the number of available C–H reactive sites. In all cases, a range of oxidised derivatives were formed.

The reactivity of CB[*n*] was also investigated as part of a complex mixture, whereby all CB[*n*] (n = 6 – 8) were reacted in equimolar amounts. Not only did this prove the sensitivity and scope of this methodology to detect and quantify CB[*n*] (n = 6 – 8) and their functionalised derivatives simultaneously, it also showed that the distribution could be modulated. This was specifically interesting for CB[8], where mixtures containing CB[8], CB[8]-$(OH)_1$ and CB[8]-$(OH)_2$ were accessible for the first time. The controlled formation of mixtures with a narrow range of oxidation products is a new approach to accessing f-CB[*n*].

From the results presented on both 'solo' and competitive oxidation it was clear that oxidation of CB[*n*] to CB[*n*]-$(OH)_1$ does not proceed in a stoichiometric manner. This results from CB[*n*] containing 2*n* available chemically equivalent C–H equatorial reactive sites. Furthermore, the introduction of the first hydroxyl functionality yielding CB[*n*]-$(OH)_1$ does not modulate the reactivity of any neighbouring C–H sites such that they are rendered chemically inactive. Therefore, further oxidation products (CB[*n*]-$(OH)_{>1}$) for all CB[*n*] are observed. The non-stoichiometric nature of CB[*n*] oxidation is in direct contradiction to what was reported by Bardelang, Ouari and co-workers[13] recently.

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A more detailed study with CB[7] showed the effects of the addition of a guest moiety either pre– or post–oxidation. It was found that addition of a guest before oxidation not only enhanced the solubility but also rendered the reaction much more controlled with a considerably narrower range of reaction products obtained. Indeed, through controlling the reaction conditions we were able to show a modulated oxidation of CB[7] to yield a mixture containing unreacted CB[7], CB[*n*]-$(OH)_1$ and trace amounts of CB[*n*]-$(OH)_2$. Through computation studies we were able to unveil that this second OH substituent showed a marked preference to be located on the same glycoluril unit as the first. This supports our results showing that stopping the oxidation at a monofunctional CB[*n*] is not possible as the introduction of the first OH has an activating effect on its adjacent position. Moreover, the presence of the second OH substituent on the same glycoluril unit renders it as a 'monofunctional CB[7]' as, on account of sterics, only one OH group can undergo further transformations and conjugations. This facile, scaleable approach to the formation of monofuncitonal CB[7] offers a new route to access monofunctionalised CB[*n*], a long term aim within the field.

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